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Improving the coverage and accuracy of syphilis testing: The development of a novel rapid, point-of-care test for confirmatory testing of active syphilis infection and its early evaluation in China and South Africa

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ABSTRACT

Background: Current point-of-care tests (POCT) for syphilis, based on the detection of *Treponema pallidum* (TP) total antibodies, have limited capacity in distinguishing between active and past/treated syphilis. We report the development and early evaluation of a new prototype POCT based on the detection of TP-IgA antibodies, a novel biomarker for active syphilis.

Methods: The TP-IgA POCT (index test) was developed in response to the World Health Organisation (WHO) target product profile (TPP) for a POCT for confirmatory syphilis testing. Two sub-studies were conducted consecutively using 458 pre-characterised stored plasma samples in China (sub-study one, addressing the criteria for the WHO TPP), and 503 venous blood samples collected from pregnant/postpartum women in South Africa (sub-study two, addressing potential clinical utility). Performance of the index test was assessed against standard laboratory-based serology using a combination of treponemal (TPHA) and non-treponemal (rapid plasma reagin [RPR]) tests.

Findings: In sub-study one, the index test demonstrated 96.1% (95%CI=91.7%-98.5%) sensitivity and 84.7% (95%CI=80.15–88.6%) specificity for identification of active syphilis (TPHA positive, RPR positive). It correctly identified 71% (107/150) samples of past-treated syphilis (TPHA positive, RPR negative). In sub-study two, the index test achieved 100% (95%CI=59%-100%) sensitivity for active syphilis and correctly identified all nine women with past syphilis.

Interpretation: The TP-IgA POCT has met the WHO TPP for a POCT for diagnosis of active syphilis and demonstrated its potential utility in a clinical setting. Future studies are warranted to evaluate field performance of the final manufactured test.

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Research in context

Evidence before this study

Maternal and congenital syphilis has re-emerged as a global health challenge. In resource-constrained settings, limited availability and lack of access to confirmatory testing of active infection constrain efforts to prevent congenital syphilis. We searched PubMed to identify peer-reviewed articles reporting performance of POCTs that can be used for confirmatory testing of active syphilis. We found that the Dual Path Platform (DPP) Screen & Confirm Assay is the only POCT with field evaluation data but has limited capacity in distinguishing between active and past/treated syphilis.

Added value of this study

This is the first study to assess diagnostic performance and clinical utility of a newly developed TP-IgA POCT for confirmatory syphilis testing. Results of this study provide empirical evidence showing that when used in combination with standard TP screening methods, the new confirmatory test has capacity to identify past syphilis whilst maintaining a high sensitivity for active syphilis infections compared to other POCTs on the market. This finding represents a significant development in the POC diagnostic landscape for syphilis, supporting the global effort in prevention of mother to child transmission and elimination of congenital syphilis in settings where laboratory capacity is limited.

Implication of all the available evidence

Data from the WHO global sexually transmitted infection surveillance suggest that the global targets for screening and treatment of syphilis among pregnant women are not being met with only 18/194 (9%) of countries have achieved at least 95% of pregnant women screened for syphilis and 95% who tested positive receiving treatment. Low and middle-income countries (LMICs) with limited resources for health face the greatest challenges. Current, commonly used rapid POCTs for syphilis have limited capacity in distinguishing between active and past infections, leading to over-treatment and further strains on health care resources. The availability of the TP-IgA POCT as a confirmatory test for women initially reactive with a screening POCT will provide more options for LMICs to enhance effective and efficient use of limited health system resources for screening, diagnosis and treatment of active syphilis among pregnant women.

Treatment of syphilis is relatively simple, effective and inexpensive with one dose of Benzathine penicillin [3]. However, diagnosis of active syphilis, particularly in LMICs, is often challenging due to the persistence of IgG antibodies to TP after prior treated syphilis infections, which necessitates a combination of treponemal (e.g. TPHA, TPPA) and non-treponemal (e.g. RPR) tests to distinguish active from past treated infections. Availability and introduction of point-of-care tests (POCTs) has increased access to testing for syphilis; however, current POCTs for serological diagnosis of syphilis only detect IgG or both IgG and IgM antibodies specific to TP. It is known that serological tests based on IgG cannot distinguish between active and treated infections, and IgM tests can be highly sensitive in symptomatic patients but have suboptimal sensitivity in asymptomatic cases [4].

The Dual Path Platform (DPP) Syphilis Screen & Confirm test (ChemBio Diagnostics, Medford, NY, USA) is the first commercially available rapid POCT that gives both treponemal and non-treponemal results that assist in distinguishing between current and past infection [5]. However, marked variation in diagnostic performance of the test has been reported particularly in the sensitivity and specificity of the non-treponemal component [5,6]. A laboratory evaluation in Australia [7] reported that 49.8% (105/211) presumptive past treated serum samples were misclassified as active syphilis by the DPP test, whereas a field evaluation among pregnant women in Burkina Faso [8] showed 48.4% (44/91) women with active syphilis would be undiagnosed had the DPP test been used. The difference in test performance can be partly explained by the lot-to-lot variation as a laboratory study in the United States [9] showed a significant difference in the sensitivity for the non-treponemal component of the DPP in two different lots studied - 65.3% (95%CI: 60.5–69.8) and 80.9% (95%CI: 72.6–87.2) respectively.

Given that the re-emergence of a syphilis epidemic among pregnant women and key populations is a global health concern [10], innovative assays using a biomarker that can accurately distinguish between past, treated and active infection at the point-of-care (POC) are needed. Access to such an assay will improve diagnosis and facilitate appropriate treatment in a timely manner [11]. To promote research and development of reliable, low-cost POCTs for syphilis, the WHO developed a Target Product Profile (TPP) identifying minimum and preferred assay performance for screening and confirmation of active syphilis (and other STIs) [12]. The TPP specifies a minimum clinical sensitivity of >80% for patients with high-titre RPR (≥ 8) and specificity of >80%, with optimal performance of >90% and >95%, respectively, for screening and confirmation of syphilis infection. The WHO TPP also highlighted the need for identification of new markers for acute syphilis infection [12]. With these needs in mind, researchers at the Burnet Institute developed a novel POCT for diagnosis of active syphilis infection based on detection of TP-specific IgA antibodies.

Whilst TP-IgG/IgM antibodies have shown some promise as a biomarker for acute syphilis infection, TP-IgA has not been systematically evaluated before. Our laboratory experiments using enzyme-linked immunosorbent assays (ELISAs) with confirmed active syphilis and past syphilis samples revealed two observations that have led to development of a more sensitive and specific POCT. Firstly, TP-specific IgA had higher reactivity rates than IgM in active syphilis samples. Secondly, inclusion of a novel TP antigen (TP0453) [13] along with the conventional antigens used in most POCTs further boosted IgA sensitivity, reaching the WHO TPP for confirmation of active syphilis. These reagents were subsequently incorporated into the TP-IgA POCT. During evaluation of early test prototypes using 79 reference plasma samples from the National Serology Reference Laboratory (Melbourne, Australia) the TP-IgA POCT achieved 91.2% sensitivity (31/34) for target active syphilis samples, 76% specificity (19/25) for samples of past-treated syphilis, and 95% (19/20) specificity for healthy control (no syphilis) samples, potentially meeting the WHO TPP (unpublished data).

1. Introduction

Syphilis is a curable sexually transmitted infection (STI), caused by the bacterium *Treponema pallidum* (TP). Globally, an estimated six million new cases of syphilis are diagnosed annually, most of the infections occur in low and middle-income countries (LMICs) and congenital syphilis is not uncommon [1]. An analysis on trend over time of syphilis in 132 countries showed that between 2012 and 2016, the prevalence of active syphilis in women of reproductive age (15 to 49 years) had increased in 78 countries, of which 10 (13%) were substantive ($\geq 10\%$ proportionally, $\geq 0.10\%$ percentage-point absolute difference and non-overlapping 95% confidence intervals in 2012 and 2016) whilst among 54 countries with observed decreased prevalence only five (9%) countries experienced a substantive decrease [2].

In this study we sought to evaluate the performance of the final prototype TP-IgA POCT for detection and differentiation of active syphilis, firstly in a reference laboratory setting in China to determine test performance compared with the WHO TPP, and secondly in an antenatal clinical setting in South Africa to address potential clinical utility of the test.

2. Methods

2.1. Test under evaluation

The TP-IgA POCT is a lateral flow, in-house manufactured immunochromatographic test designed to detect TP-specific IgA class antibodies. The test incorporates a mixture of TP chimeric Recombinant antigen (TpN15, TpN17, TpN47; Fapon, China) and Tp0453 antigen (Meridian Life Sciences, USA). It uses 5 µl of specimen (whole blood, plasma or serum), which flows through a membrane where TP-specific IgA antibodies from the specimen binds with treponemal antigens immobilised on the membrane as a line in the test area marked "Test line". Other IgA antibodies (along with other sample constituents) continue migrating along the membrane and immunoglobulins of IgG, IgM and IgA class (if present) bind with immobilised protein L in the control line area marked "Control line". Anti-human IgA colloidal gold is rehydrated and migrates past the treponemal-specific IgA bound to the antigens immobilised in the test line area, leading to the formation of a pink to purple line which confirms a positive test result. The absence of this line indicates a negative result. Unbound anti-human IgA gold continues to migrate to the IgA bound to the control line area, forming a deep pink to purple line. Absence of the control line indicates an invalid result (if the test has not been performed properly) or the presence of IgA deficiency in the patient. The total testing procedure should take 30 min. (Fig. 1)

2.2. Study design

Two sub-studies were conducted consecutively at the National Centre for STD Control in China (NCSC) and the Rahima Moosa Mother and Child hospital (RMMCH) in South Africa to address the stated objectives. The NCSC is part of an international network of clinical and laboratory-based evaluations of sexually transmitted infection tests. The Centre had experience in laboratory evaluations and development of recommendations for use of several approved rapid diagnostic test for syphilis in China and other countries. The RMMCH is an academic, regional hospital affiliated with the University of Witwatersrand. In 2018, there were an average of 1075 babies born per month, with a perinatal mortality rate of 28.9/1000 births.

2.2.1. Sub-study 1 - A retrospective laboratory evaluation of diagnostic accuracy using stored plasma samples in China

Specimen collection: Between June-December 2017, pre-characterised stored plasma samples were retrieved from the NCSC laboratory in Nanjing, China. The samples were selected on the basis of treponemal (TPHA) and non-treponemal (RPR) reactivity for diagnosis of active syphilis as specified in the WHO TPP. Three sample types were selected: (i) active syphilis (TPHA positive, RPR ≥8); (ii) past treated (TPHA positive, RPR negative); and (iii) no syphilis (TPHA negative, RPR negative). We obtained ethics approval from the Alfred Hospital ethics committee in Melbourne (AHEC #220/15) and the Institute of Dermatology, Chinese Academy of Medical Science (#2016-016).

Reference tests: Selected samples stored at −80 °C were thawed and underwent confirmatory testing with TPHA and RPR laboratory-based reference tests following the manufacturer's instructions to confirm pre-characterized syphilis status. Discordant results were retested and samples that remained discordant upon retesting were excluded and additional samples were requested. Only samples with

Technology under development: A Visual, lateral flow TP-IgA POCT

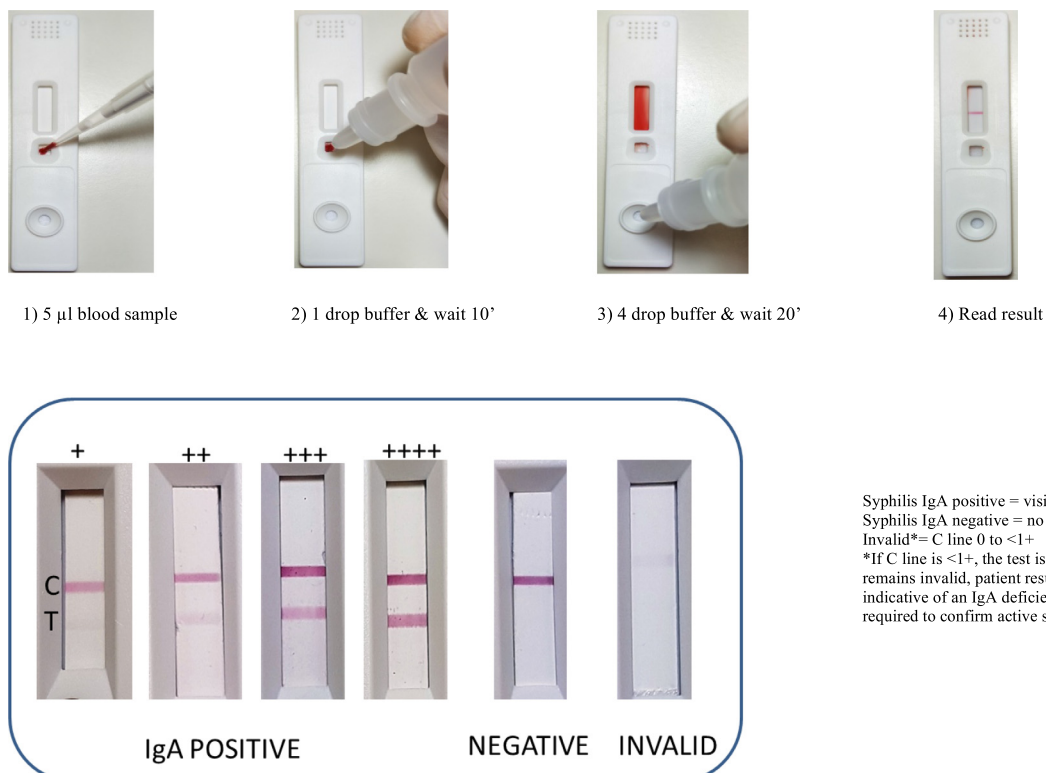


Fig. 1. Testing procedure and result window of TP-IgA POCT.

concordant confirmatory test results for both treponemal and non-treponemal components were included in the final sample panel used for the study.

Testing procedure: After confirmatory testing, each sample of the final sample panel was tested with three different syphilis POCTs: the TP-IgA POCT (index test), and two syphilis total antibody tests – the Alere Determine™ Syphilis TP test (Abbott Diagnostics, UK) and the Visitect® Syphilis test (Omega Diagnostics, UK). The two latter tests were selected because these were among the most commonly used rapid POCT for syphilis with good diagnostic performance[14] and we could not obtain the DPP Syphilis Screen & Confirm test for this study. All tests were performed by laboratory technicians following manufacturer's instructions; results were read and recorded independently of each other.

2.2.2. Sub-study 2 – a prospective evaluation of potential clinical utility using fresh venous blood samples in South Africa

Specimen collection: Between June–December 2018, venous blood samples were consecutively collected from consenting pregnant or peripartum women (within two weeks of miscarriage, termination of pregnancy or childbirth), aged 18 years or older, attending obstetric and gynaecology department at the RMMCH in Johannesburg, South Africa. Each participant provided 3 ml venous blood collected in EDTA tubes for standard syphilis serology (reference) tests and the index test performed in duplicate by two independent test operators. Ethics approval was obtained from AHEC (#556/17) and the Human Research Ethics Committee of the University of Witwatersrand in South Africa (#R14/49). All participants provided signed informed consent.

Reference tests: A combination of a treponemal test (TPHA) and a non-treponemal test (RPR) was used as standard laboratory-based reference testing for diagnosis of active syphilis infection. For the treponemal component, the ADVIA Centaur Syphilis assay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) was used and for the non-treponemal component, we used the RPR (Omega Diagnostics, UK) test. As per standard of care at the study site, a woman who had reactive results for both TPHA and RPR tests (any titre) was defined as having active syphilis; a reactive TPHA and non-reactive RPR indicate past/treated syphilis; a non-reactive TPHA and a reactive RPR is biologically false positive; and non-reactive results to both tests is no syphilis.

Testing procedure: All blood specimens were processed within 24 h of venepuncture by standard reference and the TP-IgA POCT assays. The index tests were performed in duplicate by two independent trained laboratory technicians at the RMMCH's research laboratory. Results of the index test were obtained and recorded in a blinded fashion, independent from each other and independent of the reference tests, which were performed at an offsite laboratory.

2.3. Statistical analysis

For sub-study 1, we used a purposively selected quota-sample of 458 specimens (154 active syphilis, 153 past-treated syphilis and 151 healthy control). This sample size would be adequate to estimate diagnostic accuracy of the TP-IgA POCT, with 95% confidence level, at the precision of estimates of 5% and 7% and expected sensitivity and specificity of 90% and 80%, respectively [15]. For sub-study 2, we aimed to test diagnostic performance of the TP-IgA POCT in identifying active syphilis using pre-determined values of 96% sensitivity and 85% specificity, based on the results of sub-study 1. We calculated that a sample size of 500 would provide 99% confidence with 90% power to detect a difference of 4% from the presumptive value of 96% for sensitivity and, 95% confidence with 80% power to detect a difference of 5% from the presumptive value of 85% for specificity [15].

Diagnostic performance of the TP-IgA POCT was assessed by calculating sensitivity, specificity, positive predictive value (PPV) and

negative predictive value (NPV) against standard syphilis serology tests. Specifically, to assess the performance of the index test as a non-treponemal confirmatory test for active syphilis, we compared TP-IgA positivity identified by naked eye with results of RPR. To ascertain the test capacity in distinguishing between active and past syphilis, we compared results of the index test to that of standard reference tests using a combination of treponemal (TPHA) and non-treponemal (RPR) tests. Performance of the TP-IgA test was also compared to that of the Determine™ and Visitect® syphilis POCTs using pre-characterised plasma samples (sub-study 1 only). Agreement between duplicate index tests was assessed using kappa statistics (sub-study 2 only). Numbers of invalid (no control line) and/or indeterminate test results (test operator cannot determine whether or not a test line was present in the test window) were reported. All data analysis was performed with Stata version 15.0.

2.4. Role of funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Sub-study 1: Diagnostic accuracy study

After confirmatory testing, the final panel consisted of 154 active syphilis (TPHA positive, RPR ≥ 8), 153 past-treated syphilis (TPHA positive, RPR negative) and 151 no syphilis (TPHA and RPR negative) samples (Table S1–Supplementary file). These samples came from an age range of 2 to 81 years (median: 37, IQR: 15, 70); 47% female and 53% male.

Tables 1 and 2 depict the overall results of the TP-IgA POCT using pre-characterised plasma samples following the WHO TPP categorisation [12]. Among 458 samples tested, four returned indeterminate TP-IgA test results, of which, three samples were characterised as past-treated (TPHA+/RPR-) and one was active syphilis (TPHA+/RPR+ ≥ 8) (Table S1). With the remaining 454 samples, when compared to RPR, the TP-IgA test demonstrated 96.1% (95%CI=91.7%–98.5%) sensitivity and 84.7% (95%CI=80.1–88.6%) specificity for identification of active syphilis infection (Table 1). Using a combination of TPHA and RPR as reference, 71% (107/150) of past syphilis samples (TPHA+/RPR-) returned a negative result with the TP-IgA whereas, as expected, 92% (133/145) and 100% (153/153) of these samples were reactive with the Visitect® and Determine™ POCTs, respectively (Table 2). All 154 active syphilis samples returned reactive results when tested with Visitect® and Determine™ POCTs. Three syphilis negative samples that were reactive with TP-IgA were non-reactive with the two screening tests (Table S1).

Table 1

Comparison of the TP-IgA POCT with RPR reference test results using pre-characterised stored plasma samples in China (N = 454).

TP-IgA	RPR reference			Analytical Performance% (95%CI)
	RPR+ ≥ 8	Negative	Total	
Positive	147	46	193	Sensitivity: 96.1% (91.7%–98.5%)
Negative	6	255	261	
Total	153	301	454*	Specificity: 84.7% (80.1%–88.6%)
				Positive Predictive Value (PPV): 76.2% (69.5%–82%)
				Negative Predictive Value (NPV): 97.7% (95.1%–99.2%)

* Four indeterminate IgA test results excluded from analysis.

Table 2

Performance of the TP-IgA POCT and two other POCTs in identifying active syphilis and distinguishing between active and past, treated syphilis using pre-characterised stored plasma samples in China ($N = 454$).

Assays/Reference	Active syphilis TPHA+/RPR+ ≥ 8	Past/treated TPHA+/RPR-	No syphilis TPHA-/RPR-	Total
TP-IgA				
Positive	147	43	3	193
Negative	6	107	148	261
Total	153*	150*	151	454
Determine™ Syphilis				
Positive	154	153	1	308
Negative	0	0	149	149
Total	154	153	150*	457
Visitect® Syphilis				
Positive	154	133	4	291
Negative	0	12	145	157
Total	154	145*	149*	448

* Four (IgA), one (Determine™) and ten (Visitect®) indeterminate rapid test results excluded from analysis.

3.2. Sub-study 2: Clinical utility study

Using fresh venous blood samples from prospectively recruited pregnant/postpartum women in South Africa ($N = 503$), the TP-IgA POCT detected all seven RPR positive samples (four RPR ≥ 8 and three RPR < 8) but also a small number of samples (3/503 or 0.6%) that were negative for RPR (Table 3).

Combining total antibody (TPHA) and RPR testing (Table 4), 5/503 (1%) subjects were diagnosed with active syphilis using the reference tests (TPHA+, RPR+). The TP-IgA test correctly identified 100% samples with active syphilis (5/5) and 99% (484/487) samples with no syphilis infection, and importantly was able to discriminate 9/9 past-treated syphilis cases (TPHA+, RPR-). The TP-IgA test detected 3/503 samples (0.6%) that were negative by reference tests and an additional two samples that were deemed biological false positives (TPHA-/RPR+) (Flowchart – Supplementary file).

The duplicate TP-IgA tests performed by separate operators delivered nearly identical results (99.8% agreement, kappa=0.95, $p < 0.0001$) with only one discordant result for which the reference tests were negative (TPHA-/RPR-) (Table S2 – Supplementary file).

Table 3

Comparison of the TP-IgA POCT with RPR reference test results using fresh venous blood samples from pregnant/postpartum women in South Africa ($N = 503$).

TP-IgA	RPR reference				Analytical Performance% (95%CI)
	Positive	Negative			
	≥ 8	< 8			
Positive	4*	3*	3	10	Sensitivity: 100% (59%–100%)
Negative	0	0	493	493	Specificity: 99.4% (98.2%–99.9%)
	4	3	496	503	PPV: 70% (34.8%–93.3%)
					NPV: 100% (99.3%–100%)

* Four samples with RPR titre ≥ 8 (one 1:8 & three 1:32); three samples with RPR titre < 8 (two 1:1 & one 1:2).

4. Discussion

In addressing the WHO TPP for a syphilis confirmatory test, the primary intended use of the TP-IgA POCT is an alternative to laboratory-based non-treponemal (RPR) testing, used in combination with a syphilis total antibody (treponemal) screening test. Results of the present studies show that the prototype TP-IgA POCT meets the WHO TPP for a confirmatory POCT for active syphilis, with clinical sensitivity and specificity greater than 95% and 80%, respectively. The TP-IgA POCT can potentially be a useful diagnostic tool for confirmation of active syphilis infections at the point-of-care in resource-constrained settings where both the availability of and access to laboratory services are limited. In addition, it has the potential to significantly reduce overtreatment by distinguishing active syphilis from past/treated syphilis when used in conjunction with a treponemal-specific total antibody test.

There are many rapid POCTs for syphilis total antibody already available in the market, including the Alere Determine™ and Vistect® devices examined here. These tests are highly sensitive, affordable and easy to use by healthcare workers in clinical settings with minimal training [14,16,17]. Yet, because these tests are based on detection of total treponemal-specific antibodies they cannot distinguish between active and past-treated infections. Use of these tests without confirmatory testing for active syphilis is not only contrary to policy in many countries, but introduces the risk of unnecessary treatment of individuals who are treponemal antibody positive due to previously treated syphilis, and non-treponemal test will be required for subsequent clinical management [17]. This overtreatment carries public health consequences as it may affect peoples' willingness to use health services (e.g. antenatal care (ANC) for pregnant women) over fear of being administered unnecessary medications and stigmatisation [18,19]. Overtreatment could also lead to drug stock-out in resource-constrained, high prevalence settings and may contribute to the global shortage of Benzathine penicillin [20].

Table 4

Performance of the TP-IgA POCT in identifying active syphilis and distinguishing between active and past treated syphilis compared to standard laboratory reference testing (TPHA/RPR) using fresh venous blood samples from pregnant/postpartum women in South Africa ($N = 503$).

TP-IgA/Reference	TPHA+/RPR+		TPHA+/ RPR-	TPHA-/RPR-	TPHA-/RPR+	Total
	RPR ≥ 8	RPR < 8				
	Active, high titre	Active, low titre	Past/ treated syphilis	No syphilis	Biological false positive	
Positive	4	1	0	3	2	10
Negative	0	0	9	484	0	493
Total	4	1	9	487	2	503

Findings from our studies have some clinical implications for potential use of the TP-IgA POCT in field settings.

First, for the diagnosis of active syphilis with high RPR titre (≥ 8), the use of TP-IgA POCT as a confirmatory test in combination with a syphilis total antibody POCT could potentially deliver 96% to 100% sensitivity. This level of sensitivity meets the WHO TPP and is similar to that of other rapid POCTs for syphilis available on the market that cannot discriminate past/treated from active syphilis [21]. Diagnosis of active syphilis, however, could be challenging due to variation in patient's clinical manifestations and immune responses at different stages of syphilis [22]. Subjects with a positive treponemal total antibody test but negative non-treponemal (or in this case TP-IgA) test for syphilis could have past/treated syphilis, or in a small proportion could have very early syphilis, long-standing latent syphilis or active syphilis with negative non-treponemal test [23]. Those latter cases could potentially be detected using a second but different treponemal test, and those with two positive treponemal test results and no history of treated syphilis are candidates for treatment [11,24]. Although clinics in resource-constrained settings may find this challenging, it could still be worth doing given the risk and potential associated cost of missing cases of untreated infection. Results from our study in China indicated that six out of 153 active syphilis cases would have been missed and three out of 151 "no syphilis" cases would have been unnecessarily treated if only the TP-IgA POCT had been used. However, if used together with other rapid tests the first six cases would be deemed as requiring treatment and the later three cases would be deemed biological false positives. We therefore suggest that the TP-IgA POCT would be most suitable for use in combination with a standard rapid treponemal total antibody test for diagnosis of active syphilis infections in clinic settings. This would be an effective and efficient testing algorithm in settings with limited laboratory capacity. (Fig. 2)

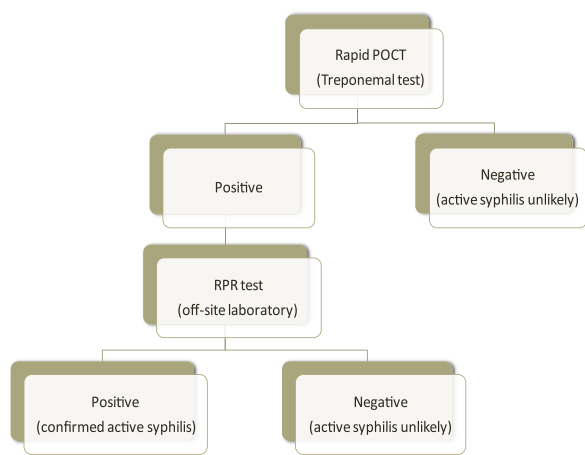
Second, the diagnosis of syphilis infection with low RPR titre (TPHA/TPPA +, RPR < 8) would be of clinical interest. Although this serological category is not included in the WHO TPP because RPR remains stable at low titre in many cases of successfully treated syphilis (serofast reaction), clinical guidelines in many countries dictate that all patients reactive to both treponemal (TPHA or TPPA) and non-treponemal tests (RPR) at any titre are considered to have active

syphilis and should be treated. Results of our South Africa study suggested that the TP-IgA POCT could potentially be sensitive enough to pick up syphilis cases with low RPR titre, enabling same day testing and treatment at the point-of-care. In addition, the use of the TP-IgA test in combination with another standard screening test would further identify 71% of past-treated syphilis, significantly reducing over-treatment. It is noted that the DPP Syphilis Screen & Confirm test, although meeting the WHO TPP with a sensitivity of > 85% for detecting samples with high RPR titre (≥ 8), does not appear to reduce over treatment of syphilis [7,8].

Third, current treponemal serological tests for syphilis use native or recombinant *Treponema pallidum* antigens to detect IgM and IgG antibodies to treponemal components [25,26]. They are more sensitive than non-treponemal tests for all stages of syphilis but cannot distinguish active from past-treated infection and therefore, cannot be used to monitor the effectiveness of syphilis treatment. Our study findings showed that the TP-IgA POCT is highly sensitive, able to distinguish between active and past infections and can potentially be used for monitoring treatment outcomes. It will also be of interest to determine the persistence of IgA antibodies in patients with non-venereal treponemal infections such as non-venereal trepanematoses, which may confound results especially in young subjects from endemic regions [8]. These particular applications of the TP-IgA POCT, however, need to be further examined in prospective, longitudinal field studies with a final manufactured version of the device. Future studies should also evaluate diagnostic performance of the test for congenital syphilis and in individuals who have become reinfected as people with repeated episodes of syphilis (e.g. key population at high risk of STIs such as men who have sex with men) may have different immunological responses compared to those with initial infection [27].

The present studies have some limitations. First, for the China study we were unable to source the DPP tests to perform a head-to-head comparison with the TP-IgA POCT in identifying active and/or past/treated syphilis from a pool of pre-characterised stored plasma samples as originally planned. This makes us unable to draw conclusion on the potential advantage of the TP-IgA POCT compared to the DPP test in distinguishing between active syphilis and past/treated infections. Future study to directly compare the two tests for this

Standard reverse algorithm with an RPR laboratory confirmatory test



Reverse algorithm with on-site confirmatory TP-IgA POCT

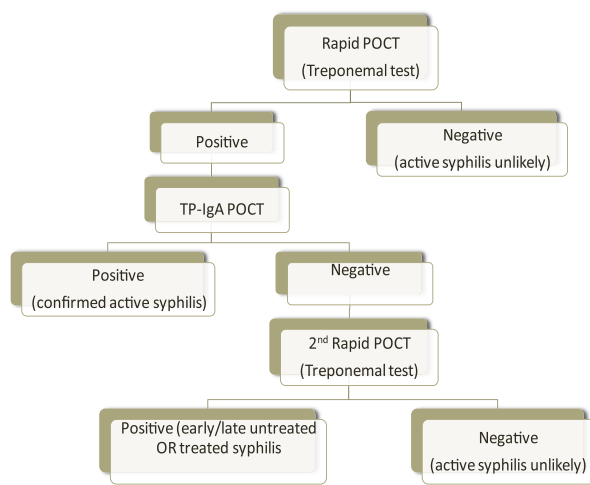


Fig. 2. Standard laboratory-based and proposed Point-of-care testing algorithms for diagnosis of active syphilis among pregnant women.

purpose is needed. Second, for the South Africa study, there was a low prevalence of active and/or past/treated syphilis among the study population and no clinical data were collected so that the categorisation of syphilis status was solely based on serological tests, which introduces some uncertainty and limits the generalisation of study findings. Third, both studies were conducted in a laboratory environment with trained technical staff and using venous blood (either fresh or plasma) rather than finger-prick samples that would be more likely to be used in field settings. Therefore, future studies are needed to examine diagnostic accuracy of the TP-IgA POCT, as performed by health care worker in clinical settings, with different sample types.

In conclusion, our evaluation studies showed that the prototype TP-IgA has met the WHO TPP performance criteria for a POCT for confirmatory diagnosis of active syphilis infections. The test demonstrated its potential utility as an accurate confirmatory test when used in combination with a treponemal total antibody-screening test. Further study is warranted to assess diagnostic performance of the final manufactured test as well as its clinical utility in real world settings.

Declaration of Competing Interest

MLG, HV, DAA and SZ developed the syphilis IgA test at the Burnet Institute in Australia and DAA is also employed at Nanjing BioPoint Diagnostics, a spinoff company of the Burnet Institute, which will manufacture the final syphilis IgA POCT device. MLG, HV, DAA, SZ, MDP, YM and SL report grants from Saving Lives at Birth - A Grand Challenge for Development managed by USAID (award number AID-OAA-F-13-00,011) and from the Thrasher Research Fund via a project grant (award number: 12617). AW and KT report funding from the Burnet Institute, Australia, during the conduct of the study. MDP, YM, SL work at the Burnet Institute but were not involved in the development of the test. All other authors report no conflict.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2020.100440.

References

- [1] WHO. Report on global sexually transmitted infection surveillance 2018. Geneva, Switzerland: The World Health Organization; 2018. Available at <https://www.who.int/reproductivehealth/publications/stis-surveillance-2018/en/> Accessed 6th September 2019.
- [2] Korenromp EL, Mahiane SG, Nagelkerke N, et al. Syphilis prevalence trends in adult women in 132 countries - estimations using the Spectrum Sexually Transmitted Infections model. *Sci Rep* 2018;8:11503.
- [3] Hook 3rd. EW. Syphilis. *Lancet* 2017;389:1550-7.
- [4] Workowski KA, Bolan GA, Centers for Disease C and Prevention. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep*. 2015;64:1-137.
- [5] Castro AR, Esfandiari J, Kumar S, et al. Novel point-of-care test for simultaneous detection of nontreponemal and treponemal antibodies in patients with syphilis. *J Clin Microbiol* 2010;48:4615-9.
- [6] Marks M, Yin YP, Chen XS, et al. Metaanalysis of the Performance of a combined treponemal and nontreponemal rapid diagnostic test for syphilis and yaws. *Clin Infect Dis* 2016;63:627-33.
- [7] Causer LM, Kaldor JM, Conway DP, et al. An evaluation of a novel dual treponemal/nontreponemal point-of-care test for syphilis as a tool to distinguish active from past treated infection. *Clin Infect Dis* 2015;61:184-91.
- [8] Langendorf C, Lastrucci C, Sanou-Bicaba I, Blackburn K, Koudika MH, Crucitti T. Dual screen and confirm rapid test does not reduce overtreatment of syphilis in pregnant women living in a non-venereal treponematoses endemic region: a field evaluation among antenatal care attendees in Burkina Faso. *Sex Transm Infect* 2018.
- [9] Constantine NT, Sill AM, Gudesblat E, Paulus S, Saleh A. Assessment of two rapid assays for diagnostic capability to accurately identify infection by *Treponema pallidum*. *J Appl Lab Med AACC Publ* 2017;1:346-56.
- [10] Kojima N, Klausner JD. An Update on the Global Epidemiology of Syphilis. *Curr Epidemiol Rep* 2018;5:24-38.
- [11] Peeling RW, Mabey D, Kamb ML, Chen XS, Radolf JD, Benzaken AS. Syphilis. *Nature Rev Disease Primers* 2017;3:17073.
- [12] WHO. Point-of-care tests - Target Product profiles and research questions. Geneva, Switzerland: The World Health Organization; 2015. Available at <https://www.who.int/reproductivehealth/topics/rtis/pocts/en/> accessed 26/03/2019.
- [13] Van Voorhis WC, Barrett LK, Lukehart SA, Schmidt B, Schrieffer M, Cameron CE. Serodiagnosis of syphilis: antibodies to recombinant Tp0453, Tp92, and Gpd proteins are sensitive and specific indicators of infection by *Treponema pallidum*. *J Clin Microbiol* 2003;41:3668-74.
- [14] Jafari Y, Peeling RW, Shivkumar S, Claessens C, Joseph L, Pai NP. Are *Treponema pallidum* specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? Evidence from a meta-analysis. *PLoS ONE* 2013;8:e54695.
- [15] Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. *J Biomed Inform* 2014;48:193-204.
- [16] Phang Romero Casas C, Martyn-St James M, Hamilton J, Marinho DS, Castro R, Harnan S. Rapid diagnostic test for antenatal syphilis screening in low-income and middle-income countries: a systematic review and meta-analysis. *BMJ Open* 2018;8:e018132.
- [17] Tucker JD, Bu J, Brown LB, Yin YP, Chen XS, Cohen MS. Accelerating worldwide syphilis screening through rapid testing: a systematic review. *Lancet Infect Dis* 2010;10:381-6.
- [18] Zhu WF, Lei SY, Li LJ. Hepatitis C virus infection and biological false-positive syphilis test: a single-center experience. *Hepatobiliary Pancreat Dis Int* 2011;10:399-402.
- [19] Bulliard JL, Chiolerio A. Screening and overdiagnosis: public health implications. *Public Health Rev* 2015;36:8.
- [20] Nurse-Findlay S, Taylor MM, Savage M, et al. Shortages of benzathine penicillin for prevention of mother-to-child transmission of syphilis: an evaluation from multi-country surveys and stakeholder interviews. *PLoS Med* 2017;14:e1002473.
- [21] Bazzo ML, da Motta LR, Rudolf-Oliveira RCM, et al. Evaluation of seven rapid tests for syphilis available in Brazil using defibrinated plasma panels. *Sex Transm Infect* 2017;93:S46-50.
- [22] O'Byrne P, MacPherson P. Syphilis. *BMJ (Clin Res Ed)* 2019;365:l4159.
- [23] Larsen SA, Steiner BM, Rudolph AH. Laboratory diagnosis and interpretation of tests for syphilis. *Clin Microbiol Rev* 1995;8:1-21.
- [24] Henao-Martinez AF, Johnson SC. Diagnostic tests for syphilis: new tests and new algorithms. *Neurol Clin Pract* 2014;4:114-22.
- [25] Stamm LV. Syphilis: antibiotic treatment and resistance. *Epidemiol Infect* 2015;143:1567-74.
- [26] Morshed MG, Singh AE. Recent trends in the serologic diagnosis of syphilis. *Clin Vaccine Immunol* 2015;22:137-47.
- [27] Kenyon C, Tsoumanis A, Osbak K, et al. Repeat syphilis has a different immune response compared with initial syphilis: an analysis of biomarker kinetics in two cohorts. *Sex Transm Infect* 2018;94:180-6.